THE KINETICS OF THE INHIBITION OF ANAEROBIC PHOSPHATE UPTAKE BY YEAST CAUSED BY THE LOWER FATTY ACIDS AND BY 2,4-DINITROPHENOL

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SUMMARY

It has been shown that inhibition of anaerobic phosphate uptake by fatty acids and by 2,4-dinitrophenol is not immediate but that the inhibition develops gradually in time. This rules out the possibility that these compounds interact with phosphate uptake via carrier competition.

1. INTRODUCTION

Phosphate uptake by yeast can be inhibited under anaerobic conditions by the lower fatty acids as shown by SAMSON *c.s.* (1955) and also by 2,4-dinitrophenol as stated by LEGGETT (1961). We have confirmed this recently for acetate and dinitrophenol (BORST-PAUWELS & JAGER 1969). It was shown that the inhibition is not due to an impairment of glycolysis nor to a decrease in cellular ATP concentration or to an interference of the fatty acids and dinitrophenol with the fate of the absorbed phosphate, indicating that we are dealing with an interaction of the inhibitors with the transport process. Our kinetical data could be interpreted by assuming that the inhibitors affected phosphate uptake by decreasing the concentration of a hypothetical compound "Y" being present in normal metabolizing yeast and which would be either a high-energy compound or a general anion carrier showing affinity for both phosphate and the fatty acids or dinitrophenol. When Y is a carrier then it might be expected that the inhibition is immediate after addition of the fatty acids and absence of such an immediate response would give evidence against the carrier competition hypothesis.

2. METHODS

The experiments with the yeast, *Saccharomyces cerevisiae* Delft II, were carried out anaerobically at pH 4.5 in a 0.1 M sodium succinate buffer provided with 3% glucose, 10 mM KCl and 40 mM $(NH_4)_2SO_4$. The yeast is preincubated in this mixture for one hour at 25° before addition of inhibitors or radioactive phosphate. Uptake of radioactive phosphate (^{32}P) is determined according to BORST-PAUWELS & JAGER (1969) either at 25° or at 5°. Uptake of 1-^{14}C labelled butyric acid was determined by adding this compound to a 1% w/v yeast

suspension cooled down to 5°. 3 ml. samples were rapidly withdrawn from the suspension at appropriate times and filtered by suction on a Hirsch funnel provided with a filter paper of 2 cm diameter of Schleicher and Schüll No 602 h. The yeast on the filter paper was washed once with 2 ml ice cold water during about 8 seconds in order to remove the adhering medium. Then the filter papers were extracted with 3 ml 0.2 M Na_2CO_3 at 60 °C for 4 min. Samples (0.5 ml) were assayed for radioactivity by means of liquid scintillation (BORST-PAUWELS 1968).

3. RESULTS

Studies of the kinetics of inhibition of phosphate uptake were carried out by adding the radioactive phosphate and the inhibitor together to the yeast suspension and following the time course of phosphate uptake.

It appears from *fig. 1* that a certain time is needed for development of the maximum inhibition in the case of butyrate. Increasing the concentration from 2 to 8 mM leads to a more rapid establishment of the maximum inhibition which can be obtained at the concentration investigated. A recovery of the

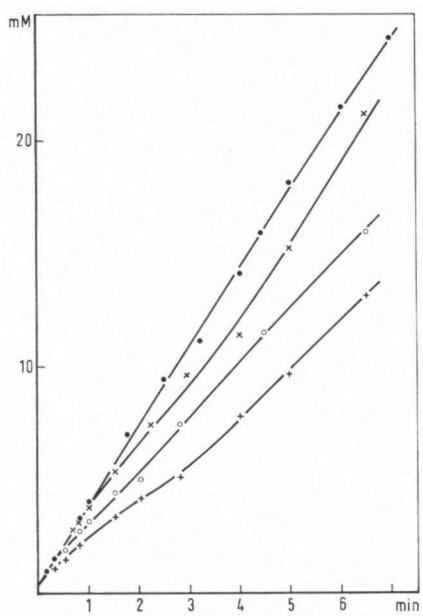

Fig. 1. Kinetics of the development of phosphate uptake inhibition caused by varying amounts of butyrate at 25°. Ordinate: radioactive phosphate concentration in the cell water. Initial phosphate concentration is 0.02 mM, the yeast concentration is 0.03 % w/v. ●: control; ×: 2 mM butyrate; ○: 4 mM butyrate and +: 8 mM butyrate.

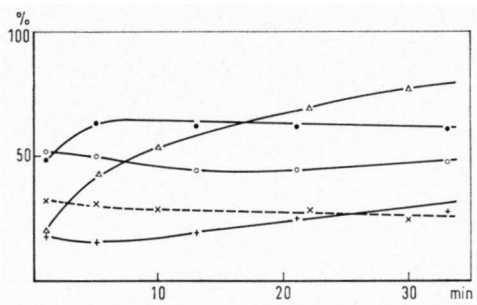

Fig. 2. Initial rates of phosphate uptake expressed in percents of the control after varying times of preincubation at 25° with different inhibitors. ●: 5mM butyrate; Δ: 20 mM acetate; ×: 0.25 mM caprylate; ○: 0.04 mM dinitrophenol; +: mM 5 formate. Initial phosphate concentration is 0.1 mM; yeast concentration is 1% w/v.

phosphate transport capacity is found after some time. The development of the phosphate uptake inhibition caused by the other fatty acids and by dinitrophenol shows also a time lag. The range of inhibitory concentrations depends upon the kind of the acid (JAGER & BORST-PAUWELS, in preparation). Also the recovery effect differs from acid to acid. This recovery is very large with acetate and somewhat smaller with formate. Preincubation of the yeast for varying time periods with acetate or formate before adding the radioactive phosphate resulted in a decrease in sensitivity of the yeast to these inhibitors as seen in *fig. 2*. Caprylate on the other hand did not give rise to a protection of the yeast and butyrate and dinitrophenol did this only to a small extent.

No delay in the inhibition of phosphate uptake is observed when adding the acids a few minutes before the radioactive phosphate. Diluting the yeast suspension five times on addition of the radioactive phosphate solution without adding extra inhibitor leads to a recovery of the phosphate transport process, as shown in *fig. 3* for the case of caprylate. The caprylate concentration in the medium decreased from 0.50 mM to 0.12 mM whereas the rate of uptake increased from 9% of the control value to 40%. The recovery occurred also with a time lag. Analogous results were obtained with the other fatty acids and with 2,4-dinitrophenol.

Preliminary experiments carried out with ^{14}C-1-labelled fatty acids revealed that the uptake of these acids into the yeast cells was completed in about the same time as the inhibition developed. Generally, however, the uptake of the acids was too rapid for a proper kinetical analysis even at 5 °C at which temperature the acids are absorbed at a rate about 20 times lower than at 25 °C. Butyrate and formate are accumulated at a somewhat slower rate than the other fatty acids are, making these acids more suitable for such a study. A comparison is made between the development of inhibition of phosphate uptake caused by addition of different amounts of butyrate and the course

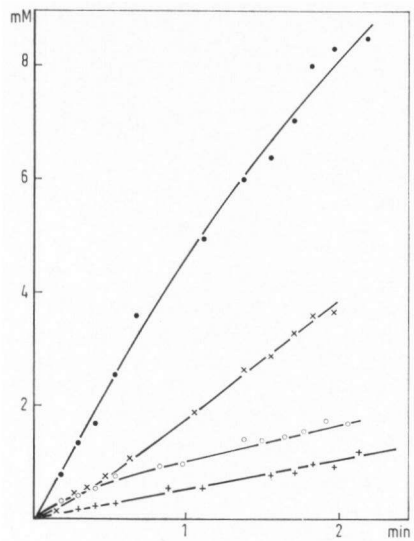

Fig. 3. Development of the inhibition of phosphate uptake caused by 0.5 mM caprylate and its reversal. Effect of a 1 min preincubation with caprylate upon the kinetics of phosphate uptake. ●: control; ○: caprylate added at zero time together with 0.1 mM radioactive phosphate; +: caprylate added 1 min prior to the phosphate; ×: caprylate added 1 min prior to the phosphate and diluted 5 times at zero time on addition of the phosphate. The final yeast concentration is 0.2% w/v.

of uptake of the acid at 5°. It is seen from *fig. 4* that the development of the inhibition of phosphate uptake is greatly retarded at 5 °C.

There is a striking parallel between the kinetics of butyrate uptake and the development of the phosphate uptake inhibition as shown by *fig. 5*. The uptake of 8 mM butyrate is almost at maximum after five minutes whereas the inhibition of the phosphate uptake is not much raised after that time. On the other hand, the gradually increasing inhibition of phosphate absorption up to 15 minutes observed after the addition of 4 mM butyrate is accompanied by an increase in the butyrate concentration of the cells.

Fig. 6 shows a plot of the rate of phosphate uptake evaluated from *fig. 4* against the cellular butyrate concentration found at varying external butyrate concentrations and at varying times of uptake. It is seen that the inhibition is almost independent of the concentration of butyrate in the medium and is mainly determined by the amount of butyrate absorbed into the cells. Similar results were obtained with formate at 5 °C. 5 or 10 mM formate did not give rise to an immediate decrease in the rate of anaerobic phosphate uptake whereas the uptake was inhibited for 50% when an internal formate concentration of about 5 mM was reached.

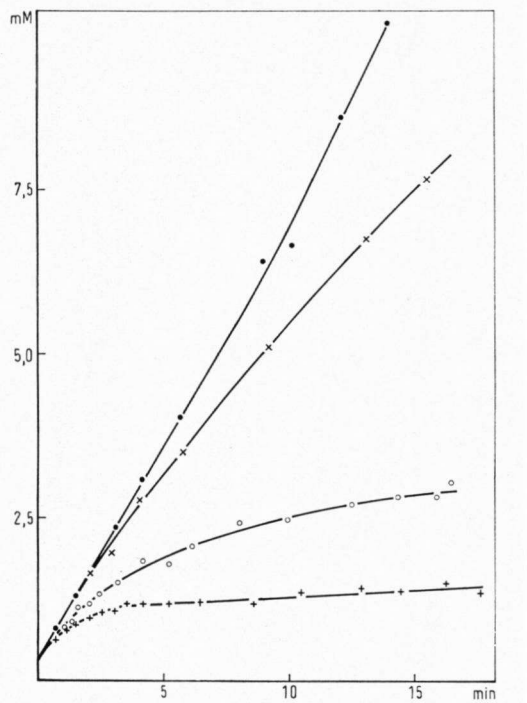

Fig. 4. Kinetics of the development of phosphate uptake inhibition caused by varying amounts of butyrate at 5°. Initial phosphate concentration is 0.15 mM, yeast concentration is 1% w/v. For further details see legend to *fig.* 1.

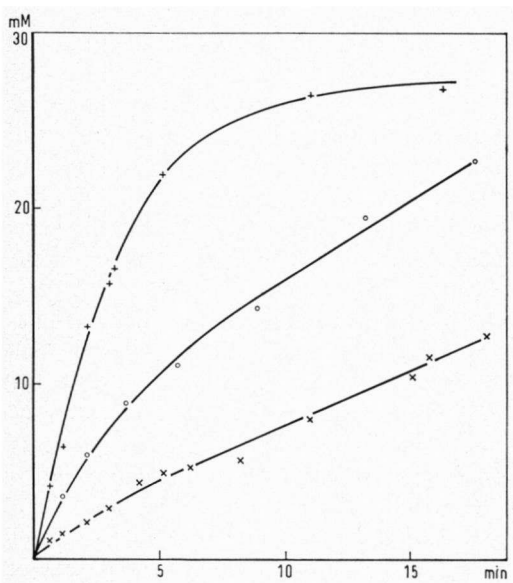

Fig. 5. Uptake isotherms of labelled butyrate in the presence of 0.15 mM phosphate at 5°. Same symbols as used in *fig.* 4.

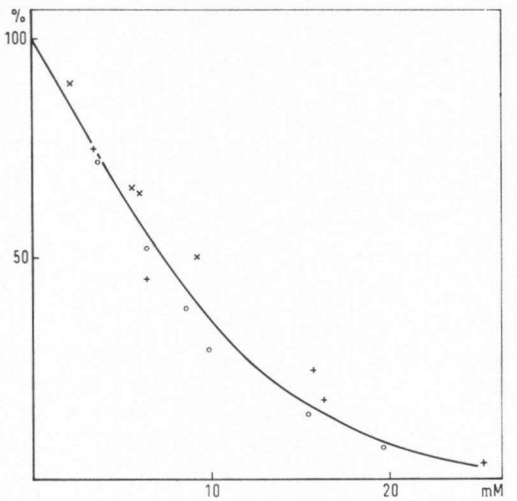

Fig. 6. Plot of phosphate uptake rates evaluated from the curves of *fig.* 4. at varying times and at varying butyrate concentrations against the corresponding internal butyrate concentrations read off from *fig.* 5.

4. DISCUSSION

The occurrence of a delay in the onset of the phosphate uptake inhibition observed when adding the inhibitory acids together with the radiaoctive phosphate indicates that accumulation of the acids into the cell is a prerequisite for their inhibitory action. This is supported by the finding of a parallel between the kinetics of entry of radioactive butyrate and the time course of the development of the inhibition found at 5 °C.

0.5 mM caprylate (*fig. 3*) caused about the same percentual inhibition as obtained with 8 mM butyrate. This is in accordance with the finding of SAMSON *c.s.* (1955) that increasing the chain length of the fatty acids leads to a greater inhibitory action of these acids.

The rapid reversibility of the inhibition may be an indication that the acids act at such and not after being converted into some other compound being the real inhibitor.

The interaction of the acidic inhibitors with the phosphate transport mechanism may be located either in the cell interior or at the inner side of the cell membrane. It has been suggested by BORST-PAUWELS & JAGER (1969) that the fatty acids and also dinitrophenol inhibit phosphate uptake by decreasing the concentration of a hypothetical compound "Y" which may be a high-energy compound as considered to be formed in yeast under anaerobic condition by both RIEMERSMA (1968) and JARETT & HENDLER (1967) or a general anion carrier as proposed to play an important role in the uncoupling of oxidative phosphorylation in animal mitochondria, see VAN DAM & SLATER (1967). The lack of

an immediate inhibition of phosphate uptake occurring on adding inhibitor and radioactive phosphate at the same time shows that the phosphate uptake inhibition is not due to a competition of orthophosphate and inhibitor anion from the medium for the same carrier site. The observation that an internal formate concentration of 5 mM causes 50% inhibition whereas 10 mM formate in the medium corresponding with an anion concentration of 9.3 mM does not inhibit phosphate uptake to a detectable degree, gives further evidence against the carrier interaction hypothesis.

The large accumulation of butyric acid into the cells is due to the fact that the cell membrane is very well permeable to the undissociated acid and not or to a much lesser extent to the anions (OURA *c.s.* 1959; KOTYK 1962). Since the cell pH is much higher than the medium pH, being about 5.9 (CONWAY & DOWNEY 1950; BORST-PAUWELS 1968) and 4.5 respectively, the dissociation of the acid will be much greater in the cell than in the medium and by this the anion concentration in the cell will be much larger than in the medium.

The mechanism by which the cell protects itself against acetate and formate is still under investigation. It is found that also the initial inhibition of anaerobic CO_2 production is diminished in time with acetate and formate.

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